Case Report

India's First Child using PGT-M, PGT-A and HLA Matching for Helping a Sibling having β-Thalassemia Major

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Abstract

β-thalassemia is a common single-gene disorder in India, with hematopoietic stem cell transplantation (HSCT) being the only cure. HSCT with matched unrelated donor is less successful, whereas finding a human leukocyte antigen (HLA)-matched related donor is difficult. Preimplantation genetic testing for monogenic diseases (PGT-M) with HLA matching is a novel option to have a matched sibling for HSCT for couples having an affected child. We present the first such case report in India. A couple, both carriers of β-thalassemia and having an affected son, underwent PGT-M with HLA matching combined with preimplantation genetic testing for aneuploidies of embryos to have a β - thalassemia-free child. This resulted in birth of a 10/10 HLA-matched sibling.

Keywords: *Human leukocyte antigen matching, hematopoietic stem cell transplantation, preimplantation genetic testing for aneuploidies, preimplantation genetic testing for monogenic diseases, β-thalassemia*

Introduction

β-thalassemia is a common single-gene disorder in India, with 30–40 million carriers and 10,000 affected children being born annually.[1] Monthly blood transfusions provide palliative cure for the affected children requiring approximately 2 million units of packed red blood cells,[2] with hematopoietic stem cell transplantation (HSCT) being curative. The probability of finding a match for HSCT for an India patient is only 0.008%.[3] Preimplantation genetic testing for monogenic diseases (PGT-M) with human leukocyte antigen (HLA) matching helps in identifying the unaffected embryos that are HLA compatible with the affected child. HSCT from such matched related donor is associated with fewer complications and better results than from a related haploidentical, mismatched unrelated, or matched unrelated donor.[4] Concurrent testing with preimplantation genetic testing for aneuploidies (PGT-A) improves the pregnancy rates.[5]

Since the first successful report of PGT-M with HLA matching for Fanconi anemia in 2001, successful use of this technique has been reported for many hematological and immunological disorders.[6]

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We report the first Indian case of a disease-free child born through *in vitro* fertilization (IVF) using PGT-M with HLA matching and PGT-A, suitable for HSCT to a sibling having β-thalassemia major.

Case Report

A couple consulted us in 2017 for having a disease-free and preferably HLA-matched child who can help in HSCT for their 4-year-old son affected with β-thalassemia major requiring monthly blood transfusions. They also have an unaffected elder daughter. The parents and their daughter were not HLA compatible for HSCT. The couple consulted a genetic counselor about the possibility of PGT-M with HLA matching. Treatment was initiated after developing a case-specific protocol using a combination of direct and indirect genetic study of HBB gene and indirect genetic study of HLA region.

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The female partner underwent three consecutive ovarian stimulations on flexible antagonist protocol using 225 IU recombinant FSH (Gonal-F, Merck-Serono, Germany), 75 IU hMG (Humog, Bharat Serum, India), antagonist (Cetrotide, 0.25 mg. Merck-Serono, Germany), and agonist trigger (Decapeptyl, 0.2 mg, Ferring, Switzerland). Three cycles of IVF treatment resulted in 18 blastocysts [Figure 1].

Trophectoderm biopsy was performed in each treatment cycle and the blastocysts were vitrified individually. Whole genome amplification was carried out with Ion SingleSeq™ kit (Thermo Fisher Scientific, MA, USA). PGT-M for β-thalassemia and HLA matching was performed using fluorescent polymerase chain reaction (PCR). The analysis of the c.93-1G >A mutation was done by a PCR amplification of mutation-containing region and subsequent genotyping by RFLP. Moreover, an indirect analysis for both c.93-1G >A and Ex1_2del mutations was performed using polymorphic markers linked to HBB gene (D11S1338, D11S1760, D11S4891, D11S2351, and D11S4181). The HLA compatibility was evaluated by analyzing several STR markers covering the entire HLA region (D6S1583, D6S1560, Tap1, D6S2443, D6S265, and MOG3). Finally, the fragment analysis was performed by capillary electrophoresis using AB3130 (Applied Biosystems, Foster City, CA, USA). PGT-A was performed using next-generation sequencing by Ion ReproSeq PGS kit (Thermo Fisher Scientific, MA, USA) on the embryos free of mutation, identified by PGT-M [Figure 2].

Single HLA-matched unaffected euploid-vitrified embryo was thawed and transferred in a hormone replacement cycle. Endometrium was prepared with estradiol valerate 8 mg and oral progesterone (dydrogesterone 20 mg/day) and vaginal progesterone (micronized progesterone 800 mg/day) added when the endometrial thickness was 9.9 mm. The selected blastocyst was transferred on February 19, 2018. The patient had a single intrauterine pregnancy, progressed normally till term and delivered a healthy baby girl (3.7 kg; APGAR: 9/9) on October 30, 2018 through elective cesarean section. Umbilical cord stem cells were cryopreserved. The baby was found to be 10/10 HLA matched with the affected sibling, and HSCT is planned once the baby is above 10 kg.

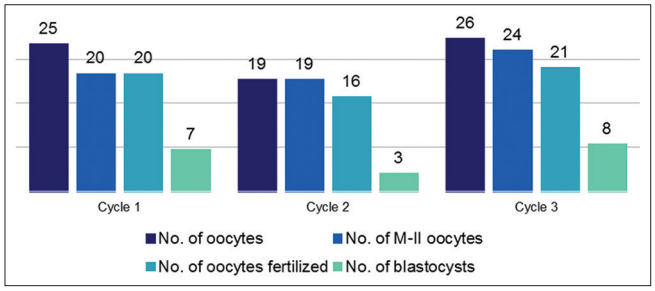

Figure 1: Ovarian response in each stimulation cycle

Discussion

Multidisciplinary care with HSCT from an HLA-matched related donor is the best curative option for children having thalassemia major; however, only 30% of patients find a suitable donor within their family.[7] HSCT with an unmatched or matched unrelated donor has high rejection and is more expensive, while with a matched sibling is associated with least complications.

The high possibility of finding an HLA-matched donor sibling with the help of IVF and PGT-M has made HSCT more cost-effective and novel treatment option. It also gives the couples an opportunity to have a healthy child and avoid abortions after prenatal diagnostic testing.

An ESHRE study from 14 centers across Europe reports 704 cycles with PGT-M and HLA matching leading to 136 live births. Of these, 57 hCT were performed and 77.3% cases that were available for follow-up reported no complications.[8] Another study by Kahraman *et al*. reports 262 cycles with 60 pregnancies and 19 siblings being cured with HSCT. Approximately 50% of cases in both these studies were for β-thalassemia.[9]

With PGT-M and HLA matching, the chance of identifying matched unaffected embryos depends on the mode of inheritance of the single-gene disorder; for a recessive autosomal or X-linked monogenic disease, it falls to 18.8%.[10]

In the ESHRE study, out of 4243 embryos analyzed for autosomal recessive diseases, 11.6% were HLA matched and unaffected.[8] Kahraman *et al*. also reported that 11.82% of the embryos were HLA matched and unaffected.[9] Our findings of 11.1% (2/18) embryos being HLA matched and unaffected are in concordance with these studies.

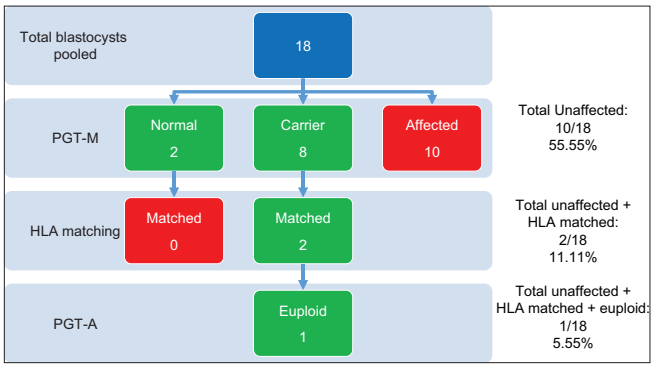

Figure 2: Step-wise analysis for finding the suitable embryo

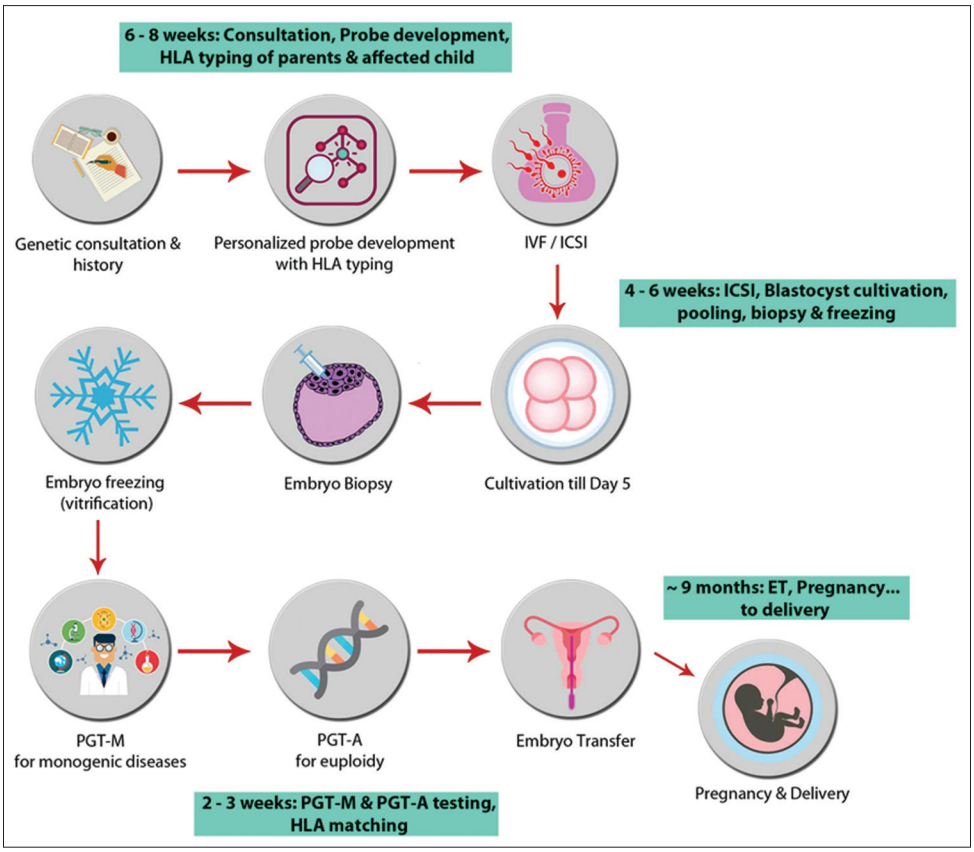

Figure 3: Outline of the sequence of events in the treatment process

Concurrent aneuploidy screening has also been recommended with PGT-M. Goldman *et al*. reported that despite having a young mean age of 32.4 + 5.9 years, only 25.6% of embryos were unaffected and euploid as analyzed by PGT-M and PGT-A.[5] We had 38.88% embryos unaffected and euploid, of these only 1 (5.55%) was HLA matched.

The entire process of having an HLA-matched, unaffected donor sibling from developing the protocol till delivery of the baby takes about 1 year [Figure 3].

IVF using PGT-M, PGT-A, and HLA matching helps in producing a disease-free child who can also help in HSCT to an affected sibling. This is the first such case reported in India. However, the low probability of finding matched euploid embryos is low and hence the need of having a high number of embryos. Awareness of such medical management should be spread in the society so that the affected children and their families could benefit without much suffering.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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